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Does dissolved organic matter or solid peat fuel anaerobic respiration in peatlands?

Anya M. Hopple[a,b,*], Laurel Pfeifer-Meister[a], Cassandra A. Zalman[b], Jason K. Keller[b], Malak M. Tfaily[c,d], Rachel M. Wilson[e], Jeffrey P. Chanton[e], Scott D. Bridgham[a]

[a] Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403, USA
[b] Schmid College of Science and Technology, Chapman University, Orange, CA 92866, USA
[c] Environmental Molecular Sciences Laboratory – Pacific Northwest National Laboratory, Richland, WA 99354, USA
[d] Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721, USA
[e] Earth, Ocean, and Atmospheric Sciences, Florida State University, Tallahassee, FL 32306, USA

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ABSTRACT

Solid-phase soil organic matter is the largest carbon (C) pool in peatlands and, as such, has long been assumed to be the primary substrate driving anaerobic respiration in these systems. However, radiocarbon data from previous field samples suggest that dissolved organic matter (DOM) plays a key, and often dominant, role in fueling heterotrophic respiration across a variety of peatlands. In this study, we manipulated available C sources under laboratory conditions to empirically determine the primary C source – solid-phase peat or DOM – fueling anaerobic respiration at surface and deep depth increments within two bogs and a poor fen in northern Minnesota. We found that increasing DOM concentration from 0 to 50% during anaerobic incubations significantly increased rates of surface (25–50 cm) methane (CH_4) production, but not carbon dioxide (CO_2) production, indicating that DOM acts as a primary driver of surface methanogenesis in peatlands. Contrary to our expectations, this response was consistent across all three sites despite differences in plant communities and biogeochemical characteristics. However, we observed no effect of DOM availability on CH_4 or CO_2 production at deeper depths (75–100 or 150–200 cm). The lack of response of CO_2 production to DOM manipulation highlights the sensitivity of surface CH_4 production to changes in the DOM pool quality and quantity, which are likely to occur under future climate change scenarios. However, CH_4 production in deeper peat appears to be limited by additional factors beyond labile C availability, at least on the timescale of the laboratory incubations.

1. Introduction

Peatlands are important terrestrial ecosystems largely due to their significant influence on the global carbon (C) cycle. In these systems, imbalances between net primary production and slow decomposition under cold, wet, and oxygen-limited conditions have led to the accumulation of about one-third of the world's soil C (Bridgham et al., 2006; Gorham, 1991) since the Last Glacial Maximum (Yu et al., 2010). Northern peatlands alone store half as much C (473–621 Pg C; Yu et al., 2010) in soil as the atmosphere stores as carbon dioxide (CO_2) (829 Pg C; Ciais et al., 2013). However, despite their immense C storage capacity, peatlands are also responsible for roughly 13% of global emissions of methane (CH_4) (Kirschke et al., 2013), a potent greenhouse gas with 45-times the sustained-flux global warming potential of CO_2 over a 100-year time frame (Neubauer and Megonigal, 2015). Given the key role these ecosystems play as both global C stores and CH_4 sources, it is imperative to gain a comprehensive understanding of peatland C cycling to elucidate current and future biosphere-climate feedbacks and mechanisms.

While solid-phase soil organic matter represents the largest C stock in peatlands, the much smaller pool of dissolved organic matter (DOM) is a key control of microbial activity (Bragazza et al., 2013) and may be particularly important in regulating the production of CH_4 in peatlands (Chanton et al., 2008; Pastor et al., 2003). Climate change could have large effects on the quality and concentration of DOM, and thus microbial activity, in peatlands. For example, changes in precipitation will affect DOM retention and transport through the peat column, both of which affect respiration of CH_4 and CO_2 (Glaser et al., 2016; Pastor et al., 2003). Additionally, studies have shown that peatland plant communities undergoing climate warming and/or drying experience

* Corresponding author at: Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403, USA.
E-mail address: ahopple@uoregon.edu (A.M. Hopple).

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decreases in the abundance of *Sphagnum* spp. and lichens and increases in the abundance of sedges and shrubs (McCalley et al., 2014; Bragazza et al., 2013; Walker et al., 2006; Weltzin et al., 2000), which may affect the type of DOM available, as well as the respiration of greenhouse gases (Wang et al., 2015; Corbett et al., 2013b). Despite the considerable attention peatlands have received, our understanding of the role of DOM in anaerobic C respiration is incomplete, limiting our ability to accurately predict climate forcing from peatlands (Bridgham et al., 2013).

Peatlands are typically classified along a hydrogeomorphic gradient of differing groundwater or surface-water inputs that influences a suite of factors, including water-table dynamics, topography, water chemistry, peat characteristics, and plant community composition. Minerotrophic fens maintain some level of groundwater connectivity and mineral-soil influence in an otherwise organic soil matrix, with porewater pH generally higher than that of bogs (CCELC, 1987). Fens with more minerotrophic features, such as higher soil pH and basic cation content, are often termed “rich”, while those with soil chemistries more similar to bogs are denoted as “poor”. Rich fens also tend to have higher nitrogen and lower phosphorus availability than that of poor fens (Kolka et al., 2016). Fen plant communities are dominated by varying proportions of graminoids, forbs, shrubs, and *Sphagnum* mosses depending on the degree of minerotrophy and hydroperiod. Conversely, extensive peat accumulation in ombrotrophic bogs, typically derived from *Sphagnum* moss, isolates the surface peat and vegetation from groundwater influence (CCELC, 1987). As a result, bog porewater pH is much lower than that of fens, with values generally ≤ 4.0 (Kolka et al., 2016; CCELC, 1987). Finally, bog plant communities typically do not contain large abundances of graminoid and forb species, but instead have greater coverage of woody shrubs and trees.

Several studies have characterized the source, reactivity, and transport of DOM with respect to vegetation and solid-phase peat along ombrotrophic-minerotrophic peatland gradients (Corbett et al., 2013a; Chanton et al., 1995; Charman et al., 1994). Radiocarbon data replicated across multiple northern peatlands from Alaska to Minnesota have linked changes in vegetation composition among bogs and fens to corresponding changes in the source of C driving heterotrophic anaerobic respiration (Chanton et al., 2008). It has been suggested that modern (recently fixed plant photosynthate, likely through root exudates) DOM is driving anaerobic respiration throughout the peat column in fens, while solid-phase peat appears to have a comparable influence over these processes in *Sphagnum*- and woody-dominated bogs. Moreover, fen DOM is generally more labile than bog DOM (Corbett et al., 2013a; Chanton et al., 2008). For example, in a comparison of bog and fen DOM chemistry in northern Minnesota, Tfaily et al. (2013) found that the majority ($> 80\%$) of surface and deep (3 m) DOM in bogs had the same chemical composition, while a considerable number of new compounds were observed in the deep fen horizon relative to surface samples, indicating greater processing of fen DOM and overall labile C limitation of decomposition at depth in both systems. Solute transport mechanisms may also affect DOM chemistry and quantity, and hence anaerobic C mineralization. For example, decadal increases in precipitation flushed surface-derived DOM deep within the peat column of bogs and fens in northern Minnesota, enriching labile C compounds and increasing the methanogenic potential at depths where microbial metabolism was likely previously limited by labile C availability (Glaser et al., 2016). The observed differences in C sources and reactivity suggest that these systems may respond to climate change very differently. These findings are also important in evaluating the stability of peat buried deep in the peatland profile (Wilson and Hopple et al., 2016). If the bulk of heterotrophic respiration in deep subsurface peat is supported by surface production conveyed to depth by DOM, then the C stored in these deeper layers is more stable than deep respiration rates alone would suggest.

Here, we investigate (i) whether DOM or solid-phase peat fuels peatland anaerobic respiration, (ii) whether this varies in two bogs and a poor fen in northern Minnesota, and (iii) if methanogenesis deep in the peatland profile is limited by the availability of surface-derived DOM. Based on radiocarbon profiles of C sources and products of respiration (Chanton et al., 2008), we hypothesized that DOM would act as a primary driver of anaerobic respiration in peatlands across depths (objective i), but that the influence of DOM would be less in the bogs (objective ii). We also expected that CH_4 production deep in the peatland profile would be stimulated by surface-derived DOM addition, and that the stimulatory response would be greater in fen vs. bog peatlands because of the greater lability of fen DOM (objective iii). While there is a body of literature consistent with our hypothesis (Glaser et al., 2016; Wilson et al., 2016; Tfaily et al., 2014; Corbett et al., 2013a; Chanton et al., 1995, 2008), these studies are primarily field observations. In this study, we conducted a direct test of the hypotheses by conducting a series of controlled incubation studies.

2. Materials and methods

2.1. Study sites

We examined the extent to which DOM or solid-phase peat fuels anaerobic respiration in three *Sphagnum*-dominated peatlands, two bogs and one poor fen, in northern Minnesota. The site characteristics of Bog Lake (BL) Fen, S1 Bog, and Zim Bog have previously been described in Medvedeff et al. (2015; and Zalman et al., 2018). BL Fen (N 47°30.304′; W 93°29.339′) and S1 Bog (N 47°30.388′; W 93°27.256′) are located within the U.S. Forest Service's Marcell Experimental Forest. S1 Bog is the site for the whole-ecosystem warming and CO_2-enrichment experiment, Spruce and Peatland Responses Under Changing Environments (SPRUCE) (Hanson et al., 2017; Wilson et al., 2016; Krassovski et al., 2015). Zim Bog (N 47°10.745′; W 92°42.877′) is ~95 km to the southeast of the other sites, within a large peatland complex that has developed in the glacial Lake Upham basin.

BL Fen is a poor fen with a soil pH of ~4.2 and a water table that is typically fairly close to the surface (−7.0 cm in July 2013 at the time of sampling; Kolka et al., 2011). It is a relatively open *Sphagnum* “lawn” with a plant community dominated by *S. papillosum* (also *S. capillifolium* and *S. magellanicum*), sedges (*Carex* spp., *Eriophorum chamissonis*), and ericaceous shrubs (*Chamaedaphne calyculata*, *Vaccinium macrocarpon*). S1 Bog and Zim Bog are both black spruce (*Picea mariana*) bogs with soil pH values ≤ 4.0, but the trees in S1 Bog are taller (5–8 m tall) and denser despite having undergone experimental strip cutting in 1969 and 1970 (Kolka et al., 2011). All sites have an average peat depth of 2–3 m.

2.2. Experimental design and sampling protocol

In June 2013, we completed a pilot experiment to investigate whether DOM or solid-phase peat is the primary driver of heterotrophic respiration in peatlands. Peat and porewater samples were collected from 25 to 50, 75 to 100, 150 to 200 cm depths at each site. Peat cores were extracted in triplicate using a Russian corer, stored on ice, and frozen within three days after transport to the University of Oregon. Two months later, we thawed the peat samples, manually homogenized replicate cores, and removed visible roots from the samples in an aerobic environment. Porewater samples were collected with a peristaltic pump from 1.25 cm-diameter PVC piezometers installed at 25, 75, and 150 cm depth increments below the hollow surface. They were then immediately frozen on dry ice and stored frozen in 500 mL polycarbonate bottles. Porewater samples were thawed, filtered through 0.7 μm glass-fiber filters (Whatman) to remove particulate C upon return to the laboratory, and re-frozen until we initiated anaerobic incubations. Peat-extracted DOM was acquired from field-moist peat samples through vacuum filtration using 0.7 μm glass-fiber filters (Whatman).

We found no difference between surface anaerobic respiration rates

from incubations using peat- or piezometer-extracted DOM (peat-extracted: $CH_4 = 0.14 \pm 0.05$ µmol C g soil^{-1} d^{-1}, $CO_2 = 2.9 \pm 0.19$ µmol C g soil^{-1} d^{-1}; piezometer-extracted: $CH_4 = 0.15 \pm 0.03$ µmol C g soil^{-1} d^{-1}, $CO_2 = 2.6 \pm 0.21$ µmol C g soil^{-1} d^{-1}). The lack of difference indicates that the two DOM sources supported comparable rates of microbial respiration, thereby justifying the use of piezometer-extracted DOM in our incubation experiments.

In July 2013, additional porewater samples were taken from piezometers at 10, 25, 50, 75, 100, 150, and 200 cm depth increments across all three sites for in-situ dissolved organic carbon (DOC) analysis. The concentration of DOC was measured by high-temperature catalytic oxidation using a Shimadzu Total Organic Carbon analyzer equipped with a non-dispersive infrared detector. Samples were analyzed in triplicate with a coefficient of variance < 2%. As an index of DOC aromaticity or humification, we measured sample specific UV absorbance (SUVA) at 254 nm and then divided the absorbance by DOC concentration (Weishaar et al., 2003). The UV absorbance was measured with a Cary Varian 100 dual beam UV/Vis spectrometer using a 10 mm Suprasil cuvette Purified Milli-Q system water was used to blank-correct sample spectra. Tfaily et al. (2013) found that SUVA was well correlated with more detailed methods of characterizing DOM aromaticity in Minnesota peatlands.

We used a variety of experimental treatments to manipulate available C sources under laboratory conditions to establish the primary substrate controlling anaerobic respiration across both different peatlands and depths. To address our principal hypothesis – that DOM acts as a primary driver of heterotrophic anaerobic respiration in peatlands, we compared the anaerobic CH_4 and CO_2 production rates in incubations with liquid phases composed of either 0 or 50% DOM (Fig. 1a). This was accomplished by first removing the majority of DOM from field-moist peat samples (protocol described below) and then creating a slurry with either deionized water (0% DOM) or piezometer-extracted porewater (50% DOM) from the same site and depth. The latter treatment is referred to as 50% DOM because manipulated peat samples were saturated with deionized water following DOM removal; thus, half of the liquid phase was deionized water and the other half was piezometer-extracted porewater. We also tested this hypothesis at DOM concentrations above 50% in surface peat by adding either deionized water or surface porewater from the same site to non-manipulated (i.e., non-washed) surface peat samples (50 and 100% DOM_{nm}; nm = non-manipulated peat) (Fig. 1b). These treatments are denoted as 50 and 100% DOM_{nm} because the 100% DOM_{nm} contains double the concentration of surface-derived DOM.

To test our secondary hypothesis – that methanogenesis deep within the peatland profile is limited by the availability of high-quality, surface-derived DOM, we added surface-derived DOM to incubations of deep (150–200 cm) peat from each site and compared rates of CH_4 and CO_2 production between treatments. We again removed the majority of DOM (see below) from field-moist peat samples collected from 150 to 200 cm within the peatland profile and then created a slurry with either surface (25 cm; 50% Surface DOM) or deep (150 cm; 50% Deep DOM) piezometer-extracted porewater from the same site (Fig. 1c). As previously stated, these treatments are referred to as 50% DOM treatments because the manipulated peat samples are saturated with deionized water following DOM removal and, therefore, contain aqueous phases composed of equal parts deionized water and porewater. If methanogenesis deep within the profile was limited by DOM quantity and/or chemistry, then CH_4 production rates should be higher with surface-derived DOM vs. deep-derived DOM. This assumes that surface-derived DOM has a higher concentration of labile C substrates than deep-derived DOM, an assumption that is justified based on previous peatland studies (see Introduction), but our experimental design does not eliminate potential effects of other surface-derived DOM properties.

Based on intriguing, but marginally significant findings (see Results) from our pilot study in June 2013 suggesting that DOM is an important driver of surface CH_4 production, we further investigated the influence of C source on peatland anaerobic respiration in July 2014 using a slightly modified experimental design and increased replication from three to five replicates to enhance our statistical power. All three sites were included in this set of experiments, and we compared the CH_4 and CO_2 production rates of peat samples with aqueous phases containing 0, 25% (a new treatment), and 50% DOM. We focused on surface (25–50 cm) and deeper (75–100 cm) peat depth increments with porewater samples collected at 25 and 100 cm (Fig. 1d). Peat and porewater sample collection and storage followed the same procedures used in June 2013, except that peat was refrigerated, not frozen, prior to laboratory analyses and incubations commenced within two weeks of field collection, not two months.

We also continued to examine our hypothesis that deep methanogenesis is limited by surface-derived DOM by repeating an experiment from June 2013 with experimental replicates increased from three to five. In this experiment, we removed the majority of DOM from deep (75–100 cm) peat samples and then added either surface (25 cm; 50% Surface 25 cm-DOM) or deep (100 cm; 50% Deep 100 cm-DOM) porewater from the same site and compared rates of CH_4 and CO_2 production between the two treatments (Fig. 1e).

2.3. DOM removal

After homogenizing specific depth increments of the three (2013) or five (2014) replicate peat cores collected at each site, samples were separated into two treatments. The majority of DOM was removed from one portion, while the other was set aside for our non-manipulated peat treatments. To remove DOM, we washed homogenized peat in mesh nylon stockings with multiple rinses of deionized water over 24–72 h. Ultraviolet absorbance at 320 nm was used to monitor the loss of DOM from sample leachate over the course of the washing using the DOC (mg C L^{-1}) and A_{320} regression equation established by Pastor et al. (2003) for northern Minnesota bogs and fens, including Zim Bog. Initial DOC concentrations ranged from 29.7 to 61.0 mg C L^{-1} for surface (25–50 cm) peat, 31.4 to 70.2 mg C L^{-1} for intermediate (75–100 cm) peat, and 18.0 to 38.9 mg C L^{-1} for deep (75–100 cm) peat. All samples were washed until DOC concentrations at A_{320} were no longer detectible. Absorbance measurements were determined on a Shimadzu UV-1700 spectrophotometer (Kyoto, Japan) at room temperature at a 10 mm path length with nanopure water serving as a blank.

Despite using a method of DOM removal that was as non-destructive as possible, we anticipated that this disturbance may affect rates of heterotrophic respiration. Thus, we compared CH_4 and CO_2 production rates between the 50% DOM and 50% DOM_{nm} samples, which were prepared differently (described above), but contained the same amount of DOM. When using frozen peat samples in June 2013, we observed a significant washing-associated disturbance effect between the 50% DOM and 50% DOM_{nm} that reduced surface CH_4 production by 56% ($p < 0.05$), but did not affect methanogenesis in deeper peat or CO_2 production (Supplemental Table 1). No washing-associated disturbance effect was observed when using fresh, non-frozen peat samples (Supplemental Table 1). While DOM removal did affect rates of surface CH_4 production in June 2013, it is not necessary for rates determined in our laboratory experiment to match those under in situ conditions to test our hypotheses. We only need to compare among treatments.

2.4. Anaerobic respiration

For all experiments, approximately 8 g of wet-weight peat were added to 120 mL serum bottles and flushed with N_2 for 15 min to create anaerobic conditions. All samples were then pre-incubated at 18 °C for three weeks to reduce terminal electron acceptors as the peat samples had been exposed to aerobic conditions for a considerable amount of time. After the pre-incubation period, peat samples were moved into a glove box filled with a N_2 atmosphere (< 5% H_2 in the presence of a palladium catalyst; Coy Laboratory Products, Grass Lake, Michigan) to

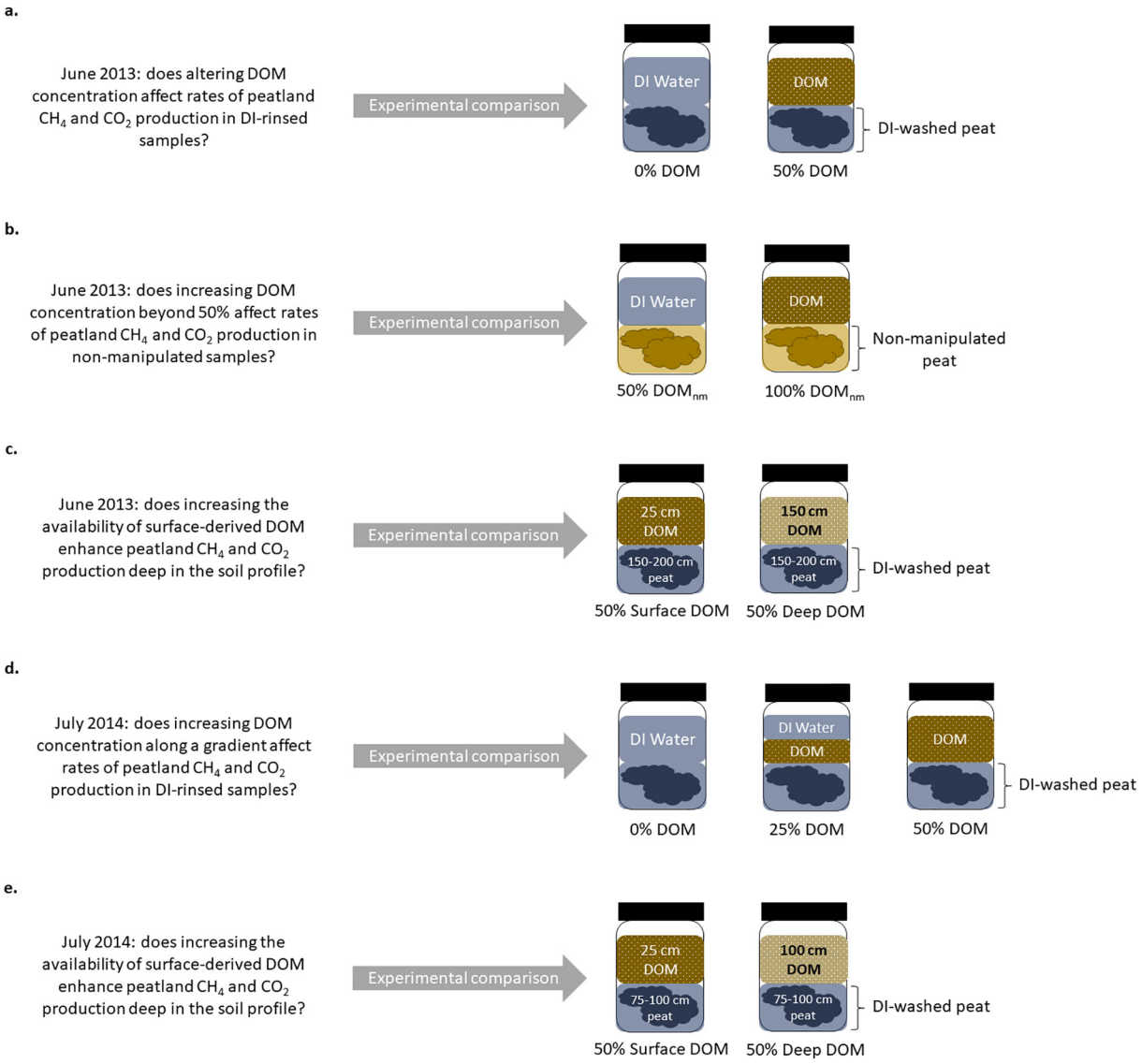

Fig. 1. Illustrative schematic of our experimental questions and corresponding laboratory treatments that manipulate available C sources during anaerobic incubations of peat samples collected from two bogs and a poor fen in northern Minnesota. Note, grey shaded peat in the figure indicates that it was rinsed of ambient DOM, while brown shading indicates it was not. In our June 2013 pilot experiment, we compared CH_4 and CO_2 production rates of (a) DI-washed peat samples with liquid phases containing 0 and 50% DOM, (b) non-manipulated peat samples with aqueous phases of 50 and 100% DOM, and (c) deep DI-washed peat samples incubated with either surface (25 cm) or deep (150 cm) DOM. Incubations were done with peat and porewater samples collected from 25 to 50, 75 to 100, and 150 to 200 cm depth increments and included three experimental replicates. Peat samples were frozen for two months prior to analysis. In July 2014, we further explored the effects of DOM on CH_4 and CO_2 production rates in anaerobic incubations of (d) DI-washed peat samples with liquid phases containing 0, 25, and 50% DOM and (e) deep DI-washed peat samples incubated with surface (25 cm) or deep (100 cm) DOM. In July 2014, incubations were completed with peat and porewater samples collected at 25–50 and 75–100 cm depths increments and included five experimental replicates. Fresh, non-frozen peat samples were used in this analysis. DI = deionized water; DOM = dissolved organic matter. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

maintain anaerobic conditions. Peat samples were brought to field moisture levels with either deoxygenated porewater or deionized water. The sample pH was adjusted to in situ pH using small aliquots of either 0.5 M HCl or 0.1 M NaOH. Sample bottles were then flushed with N_2 for 15 min to begin the incubation. Headspace samples were analyzed over the course of two weeks (days 2, 4, 7, and 13) for CH_4 and CO_2 simultaneously using an SRI gas chromatograph equipped with a methanizer and flame ionization detector. Total CH_4 and CO_2 were calculated using Henry's Law, adjusting for solubility, temperature, and pH (Bridgham and Ye, 2013). Methane and CO_2 production rates were calculated using the linear accumulation ($r^2 > 0.90$ in all cases) of gases through time.

2.5. Statistical analyses

Two-way Analysis of Variance (ANOVA) was used to determine the effects of site and treatment (as fixed effects) on CH_4 and CO_2 production rates between experimental treatments within statistically different depth increments using SPSS Statistics version 22. If no significant interaction was observed, we combined sites and analyzed differences among experimental treatments using Tukey's HSD tests ($p < 0.05$). Two-way ANOVA was also used to test for the effects of site and depth on DOC and SUVA measurements. Data were tested for normality and log-transformed where the transformation resulted in a significant improvement in overall distribution.

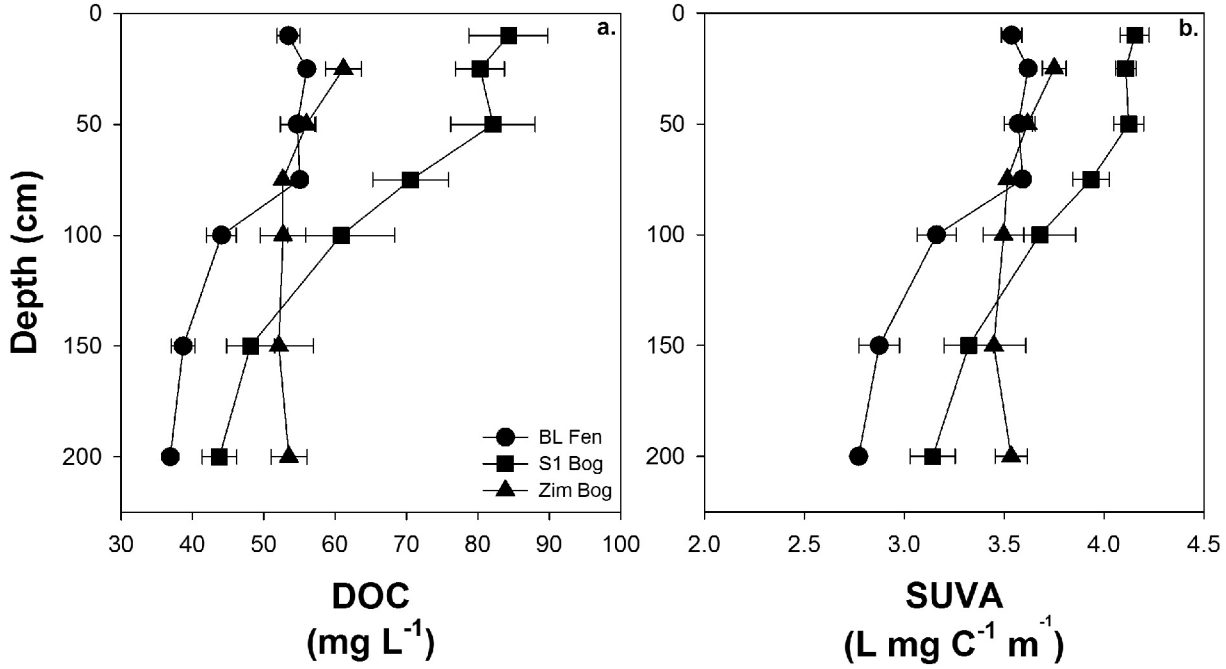

Fig. 2. Dissolved organic carbon (DOC; a) and specific UV absorbance (SUVA; b) of porewater samples collected from multiple depth increments (10–200 cm) across three northern Minnesota peatlands in July 2013.

3. Results

3.1. In-situ DOC concentration and aromaticity

In July 2013, changes in in-situ porewater DOC concentrations with depth depended on site ($p < 0.001$). DOC concentrations decreased with depth at BL Fen and S1 Bog; however, there was little change in DOC concentration throughout the peat profile at Zim Bog (Fig. 2a). Dissolved organic carbon concentrations ranged from 37 to 53 mg C L^{-1} at BL Fen, 50 to 84 mg C L^{-1} at S1 Bog, and 54 to 61 mg C L^{-1} at Zim Bog. In the surface (10–50 cm), BL Fen and Zim Bog had similar DOC concentrations (~55 mg C L^{-1}), while those of S1 Bog were approximately 33% higher (~80 mg C L^{-1}). However, at deeper depths (150–200 cm), Zim Bog exhibited the highest DOC concentrations (53 mg C L^{-1}), followed by S1 Bog and BL Fen with 46 and 38 mg C L^{-1}, respectively.

Additionally, SUVA measurements showed systematic decreases in DOC aromaticity with depth across all sites ($p < 0.001$); however, this decrease was much more muted at Zim Bog (range = 3.5–3.8 L mg C^{-1} m^{-1}) relative to BL Fen and S1 Bog (Fig. 2b). SUVA values ranged from 2.8 to 3.6 L mg C^{-1} m^{-1} and 3.1 to 4.2 L mg C^{-1} m^{-1} at BL Fen and S1 Bog, respectively. In surface peat (10–50 cm), S1 Bog had the highest aromaticity measurements (~4 L mg C^{-1} m^{-1}), while BL Fen and Zim Bog both had lower SUVA values of approximately 3.5 L mg C^{-1} m^{-1}. At depths below 100 cm, Zim Bog had the highest DOC aromaticity (3.5 L mg C^{-1} m^{-1}), followed by S1 Bog (3.2 L mg C^{-1} m^{-1}) and BL Fen (2.8 L mg C^{-1} m^{-1}).

3.2. Carbon source fueling anaerobic respiration

In our June 2013 pilot study, both CH_4 and CO_2 production decreased with depth in the peat column ($p < 0.01$, Supplemental Table 2). The interaction between site and treatment was marginally significant ($p = 0.094$), but obvious differences among sites were not evident; therefore, we analyzed CH_4 and CO_2 production responses to DOM removal and addition across all sites. Surface (25–50 cm) CH_4 production was 53% lower in incubations containing 0% DOM than in those with 50% DOM across all peatland types ($p \leq 0.07$; experimental design in Fig. 1a). However, we observed no significant DOM treatment effects on CH_4 production at any other depth, and there were no treatment effects on CO_2 production (Supplemental Table 2, $p \geq 0.12$). Additionally, surface CH_4 and CO_2 production rates did not increase when DOM concentrations were doubled from 50% to 100% in non-manipulated peat samples (50% DOM_{nm}: $CH_4 = 0.34 \pm 0.07$ μmol C g $soil^{-1}$ d^{-1} and $CO_2 = 2.3 \pm 0.13$ μmol C g $soil^{-1}$ d^{-1}; 100% DOM_{nm}: $CH_4 = 0.36 \pm 0.07$ μmol C g $soil^{-1}$ d^{-1} and $CO_2 = 2.7 \pm 0.21$ μmol C g $soil^{-1}$ d^{-1}; experimental design in Fig. 1b).

In our more replicated study from July 2014, there were highly significant site and DOM treatment effects in surface peat (site: $p < 0.01$; treatment: $p < 0.001$; experimental design in Fig. 1d), but treatment effects did not differ among sites ($p = 0.981$) for CH_4 production (Fig. 3). Across all sites, average rates of CH_4 production increased from 0.93 ± 0.28 μmol C g $soil^{-1}$ d^{-1} in the 0% DOM treatment to 1.21 ± 0.26 and 1.75 ± 0.29 μmol C g $soil^{-1}$ d^{-1} in the 25 and 50% DOM treatments, respectively, representing 30 and 88% increases in surface methanogenesis with increasing DOM content. Regardless of treatment, there was no measurable CH_4 production in anaerobic incubations of deep (75–100 cm) peat in July 2014. Consistent with June 2013 results, DOM treatment, site, and their interaction had no significant effects on surface (Fig. 4) or deep (Fig. 5) CO_2 production.

3.3. Increasing surface-derived labile carbon availability

In our June 2013 pilot study, deep peat samples incubated with surface porewater from the same site did not show increases in their rates of CH_4 production (50% Deep DOM: $CH_4 = 3.1 \pm 0.01$ nmol C g $soil^{-1}$ d^{-1} and $CO_2 = 0.85 \pm 0.043$ μmol C g $soil^{-1}$ d^{-1}; 50% Surface DOM: $CH_4 = 3.0 \pm 0.10$ nmol C g $soil^{-1}$ d^{-1} and $CO_2 = 0.88 \pm 0.075$ μmol C g $soil^{-1}$ d^{-1}; experimental design in Fig. 1c). When we repeated this experiment with increased replication in 2014, we found no measurable CH_4 production in anaerobic incubation of deep (75–100 cm) peat samples that were combined with surface (25 cm) or deep (100 cm) porewater (experimental design in Fig. 1e). Throughout this incubation, sample CH_4 concentrations were often at or below our methodological detection limits (~0.25 ppm CH_4/

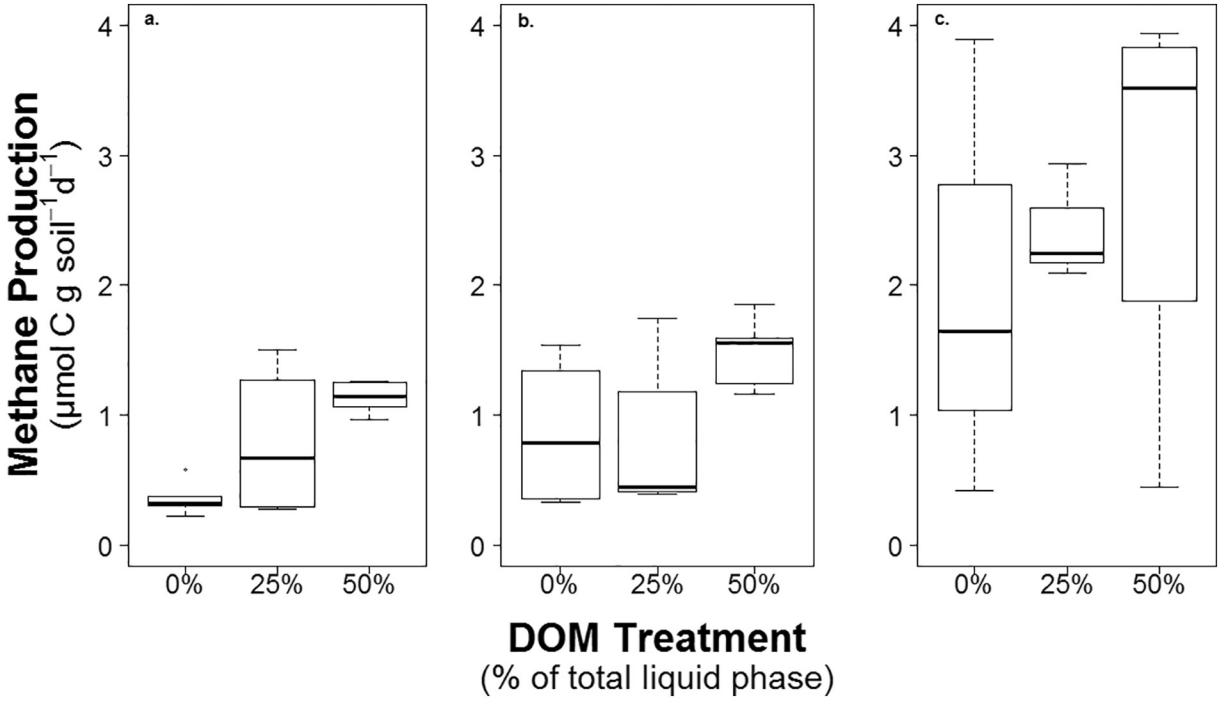

Fig. 3. Methane production in peat samples collected at 25–50 cm in July 2014 from BL Fen (a), S1 Bog (b), and Zim Bog (c). The thick black line represents the median value, the box edges denote the upper and lower 25% quartiles, and the whiskers show the maximum and minimum values (excluding outliers). Outliers are shown with open circles. DOM content significantly increased CH_4 production *across all sites* ($p < 0.001$); however, we display our results by site to demonstrate the site-specific differences in CH_4 production variability and response to increasing DOM concentrations. The experimental design is given in Fig. 1d.

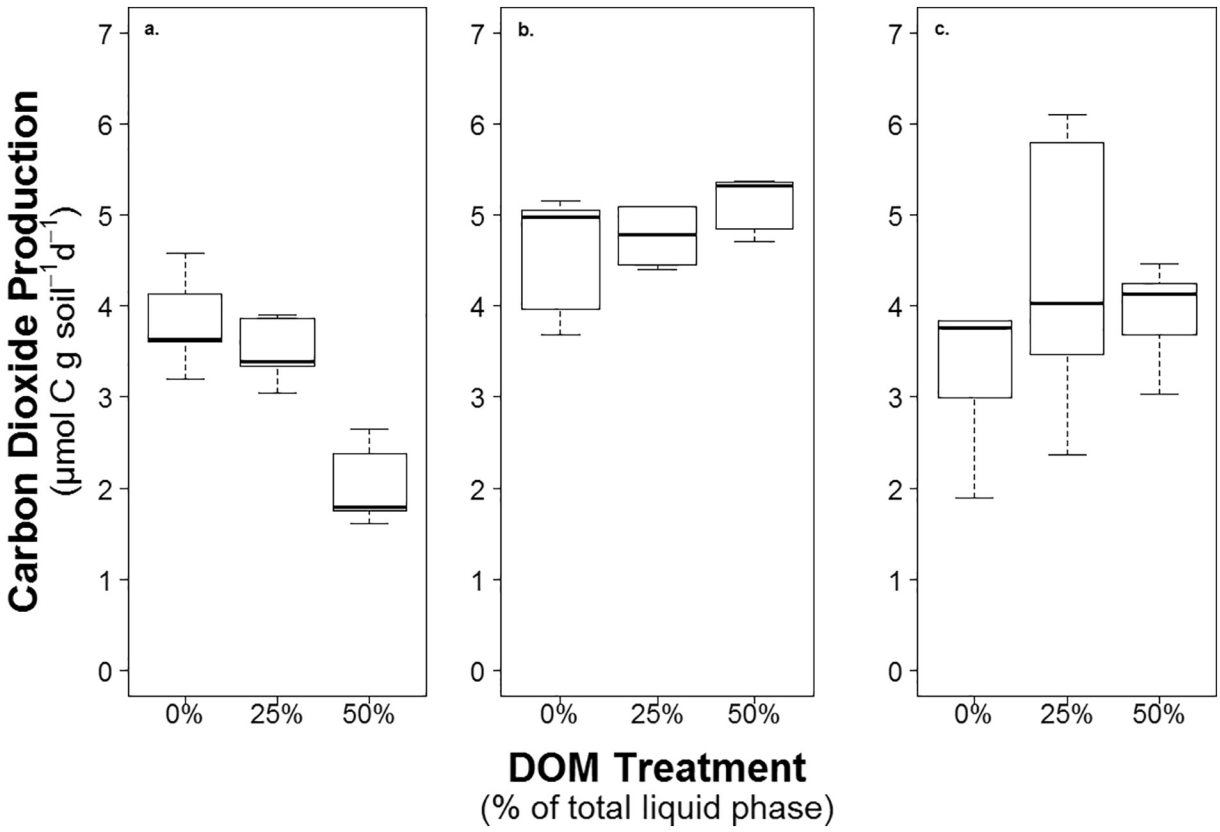

Fig. 4. Carbon dioxide production in peat samples collected at 25–50 cm in July 2014 from BL Fen (a), S1 Bog (b), and Zim Bog (c). The thick black line represents the median value, the box edges denote the upper and lower 25% quartiles, and the whiskers show the maximum and minimum values (excluding outliers). Outliers are shown with open circles. No significant differences were observed within treatments, across sites, or for their interactive effect. The experimental design is given in Fig. 1d.

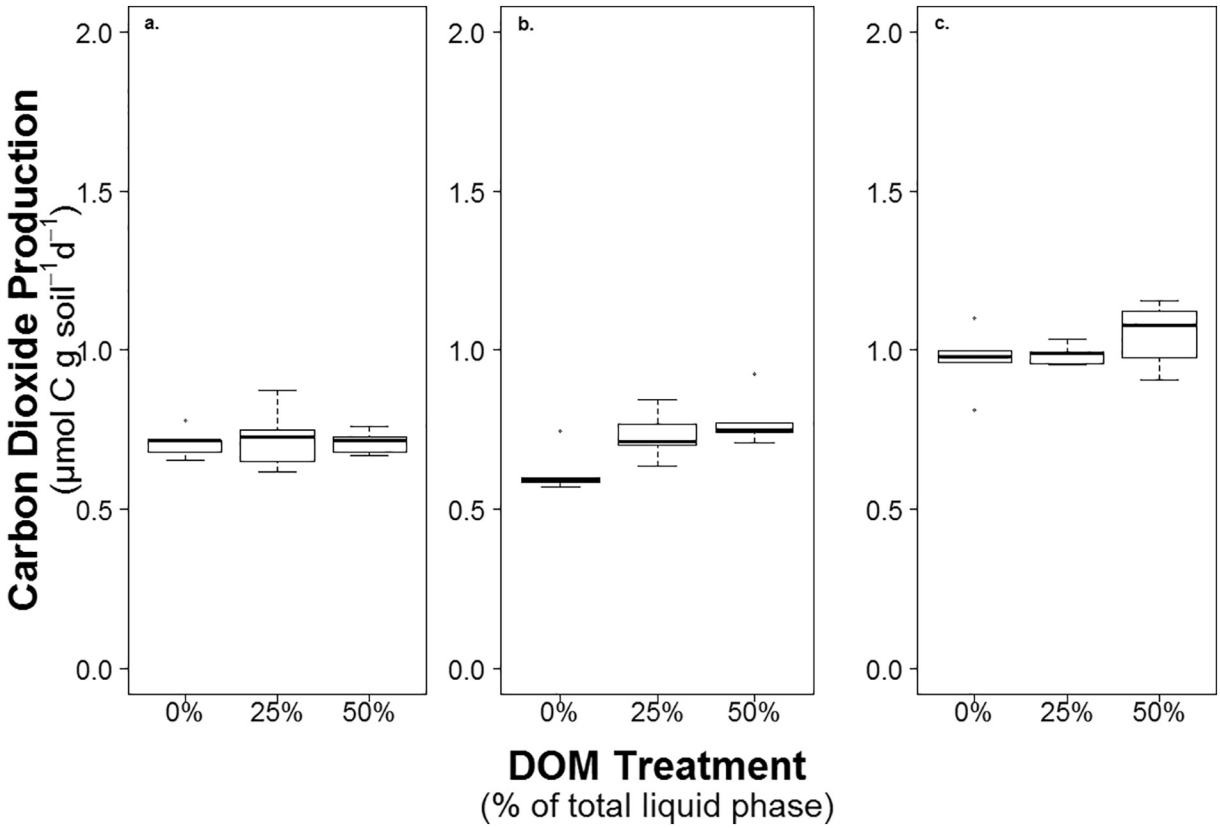

Fig. 5. Carbon dioxide production in peat samples collected at 75–100 cm in July 2014 from BL Fen (a), S1 Bog (b), and Zim Bog (c). The thick black line represents the median value, the box edges denote the upper and lower 25% quartiles, and the whiskers show the maximum and minimum values (excluding outliers). Outliers are shown with open circles. No significant differences were observed within treatment, across sites, or for their interactive effect. The experimental design is given in Fig. 1d.

mL).

4. Discussion

4.1. In-situ DOC concentration and aromaticity

DOC concentration and aromaticity decreased with depth in BL Fen and S1 Bog, suggesting that these peatlands contain reactive DOC that is utilized by microbial communities. Conversely, the lack of change in DOC concentration and aromaticity at Zim Bog indicates that this C source is fairly non-reactive. Previous studies in northern Minnesota peatlands, including S1 Bog, have shown that decreases in DOC aromaticity with depth are often associated with the formation of new compounds with low O/C and high H/C elemental ratios, indicating humification of peat and its utilization as a microbial C source (Tfaily et al., 2013, 2014). The DOC concentration and SUVA values reported here are in line with previous studies and highlight the differences in DOC quantity and chemistry across peatland types and with depth (Wilson et al., 2016; Tfaily et al., 2014; Corbett et al., 2013a; Leifeld et al., 2012; Pastor et al., 2003).

4.2. Carbon source fueling anaerobic respiration

Our experimental, laboratory-based study confirms the importance of DOM as a primary driver of methanogenesis by demonstrating that increasing DOM content significantly increased surface CH_4 production rates across all peatland types. However, we observed no effect of increasing DOM content on rates of CH_4 production when DOM was doubled from 50 to 100% in non-manipulated surface peat samples, suggesting a saturating function of DOM past 50% concentrations and indicating that some other unknown factor limits surface methanogenesis, such as microbial C use efficiency. Perhaps a longer incubation time allowing for increased methanogen population growth would have resulted in a positive response at DOM concentrations higher than 50%. This result is consistent with field studies of radiocarbon ages of CH_4, CO_2, DOM, and peat, which suggest that anaerobic respiration in peatlands is fueled by DOM (Wilson et al., 2016; Hoyt et al., 2014; Tfaily et al., 2014; Corbett et al., 2013a; Chanton et al., 2008; Chanton et al., 1995). Furthermore, a mesocosm study that incorporated warming and water-table manipulations in a Minnesota bog (monoliths were from Zim Bog) and fen found that greater DOM retention was correlated with a large increase in CH_4 and dark CO_2 flux (Pastor et al., 2003).

Overall, our results suggest that the response of methanogenesis to changes in DOM concentration is consistent across peatland type, contrary to our expectations. However, site-specific analyses provide weak evidence that fen CH_4 production may respond more strongly to increasing DOM than that of bogs. For example, when examined within each site in July 2014, the influence of DOM on CH_4 production was only statistically significant in BL Fen ($p < 0.05$), where methanogenesis increased by 54 and 68% as DOM concentration increased from 0 to 25 and 50%, respectively (Fig. 3a). There was a stepwise increase in mean CH_4 production with increasing DOM addition in Zim Bog and the highest mean CH_4 production occurred in the 50% DOM treatment in S1 Bog (Fig. 3b and c), but large variation within treatments made these effects nonsignificant.

DOM chemical quality has been cited as a main factor for controlling its use as a C substrate for microbial respiration in peatlands (e.g., Glaser et al., 2016) and was found to vary across the three peatlands included in this study (Fig. 2b). Fen DOM has a lower molecular weight,

is 3–12 times more photo-reactive, and has lower C/N ratios than that of bog DOM and, furthermore, fen DOM aromaticity decreases with depth, but aromaticity remains constant in bogs (Tfaily et al., 2013, 2014; Corbett et al., 2013a; Leifeld et al., 2012). All of these properties indicate that fen DOM is more labile and reactive than bog DOM, leading to its preferential utilization as a C source in fens relative to bogs. However, our results provide only weak evidence supporting this hypothesis, possibly because our washing process likely removed soluble compounds, such as nutrients, that could be important in controlling microbial respiration. Nevertheless, further study is necessary to better understand how different peatland types respond to changes in DOM availability and chemistry.

Field studies employing radiocarbon analyses have also found that the age of CO_2 is very similar to that of DOM in fens and intermediate between the ages of DOM and solid-phase peat in bogs – demonstrating the importance of DOM as a driver of CO_2 production in addition to CH_4 production. In contrast, we found no influence of DOM manipulation on CO_2 production rates at any site or depth in either experiment. The radiocarbon field studies were observing the net effect of production and transport pathways of gases within the peat profile that are likely temporally and spatially variable, but are measured at a single (or a few at most) point in time, which may provide different dynamics than observed in our laboratory study.

Nonetheless, it is intriguing that methanogens in our study responded to the DOM treatments in surface peat while the CO_2-producing microbial consortia did not. This study emphasizes the sensitivity of methanogenesis to differences in DOM availability and chemistry, which may be substantially altered under current and future climate change. Most peatlands occur above 40°N latitude, where the largest relative temperature changes are projected to occur under current climate models and, therefore, are perceived to be particularly susceptible to climate change (Kirtman et al., 2013). Climate warming has been shown to decrease the abundance of *Sphagnum* spp. and lichens and increase the abundance of sedges and shrubs in peatland plant communities, which could alter organic matter reactivity and humification rates. For example, Medvedeff et al. (2015) found that *Sphagnum*-derived leachates, collected from the three peatlands used in our study, played a role in regulating peatland CH_4 production and that the various *Sphagnum*-derived extracts produced responses that differed based on peatland type. Similarly, our results indicate that DOM is a major driver of peatland methanogenesis and that this process is particularly sensitive to differences in DOM availability; however, DOM measurements are not widely quantified nor incorporated into ecosystem process models or climate forcing predictions (Bridgham et al., 2013).

4.3. Increasing surface-derived labile carbon availability

While we provide evidence of the importance of DOM as a substrate for CH_4 production in surface peat, we were unable to document any effect of DOM manipulation on rates of CH_4 production in deeper peat. Although both fen and bog DOM at depth are relatively young in age compared to the solid peat matrix, only deep fen DOM (100–200 cm) has been shown to have lower lability and reactivity than surface DOM, while bog DOM exhibits similar C chemistry throughout the peat profile (Tfaily et al., 2014; Corbett et al., 2013a,b; Tfaily et al., 2013; D'Andrilli et al., 2010). Numerous studies have documented similar low CH_4 production rates in deep peat (Kotiaho et al., 2010; Cadillo-Quiroz et al., 2006; Galand et al., 2003), and we recently found that even one year of in situ warming of deep peat did not increase the low rates of methanogenesis in S1 Bog (Wilson et al., 2016). While radiocarbon data show that most anaerobic mineralization at depth is derived from relatively young DOM (Wilson et al., 2016; Tfaily et al., 2014; Chanton et al., 2008), CH_4 production is apparently proximally inhibited by unknown factors beyond temperature and substrate availability. We have observed a large decrease in gene and transcript number for the α-subunit of the methyl coenzyme M reductase (*mcrA*), a functional gene common to all methanogens, from a peak at 20 cm depth in these sites (Zalman et al., 2018), suggesting lower methanogen abundance and activity with increasing peat depth. It is likely that the methanogen population was unable to respond to increased labile DOM availability over the two-week incubation.

5. Conclusions

Traditionally, peat C mineralization is quantified on a per solid-gram basis and is used in this form to determine ecosystem respiration rates. However, peatlands have two forms of C reservoirs (as do other wetland ecosystems): solid-phase organic matter (peat) and DOM. This DOM pool is not widely incorporated into ecosystem process models or climate forcing predictions because peat has been presumed to be the major contributor of CH_4 and CO_2 emissions since it is the primary form of C storage (Bridgham et al., 2013). However, our study confirms the importance of DOM as a primary driver of methanogenesis by demonstrating that increasing DOM content significantly increased surface CH_4 production rates across a variety of peatland types. These findings generally support the conclusions of recent radiocarbon studies which suggest that anaerobic respiration in peatlands is fueled by DOM (Hoyt et al., 2014; Corbett et al., 2013a; Chanton et al., 2008). Additionally, the lack of response of CO_2 production to DOM manipulation highlights the sensitivity of CH_4 production to change in DOM quantity and quality, which are likely to occur under future climate change scenarios and emphasizes the importance of continued study into the controls of heterotrophic peatland respiration. Finally, we also show that increasing surface-derived DOM content in incubations of deep peat does not stimulate deep methanogenesis, suggesting that this process is limited by additional factors beyond labile C limitation.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2019.04.040.

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